

Amendments to the Specification

Please amend the specification as follows:

Please amend the paragraph between page 28, line 24 and page 29, line 15 as follows:

In addition to the conjugates and adjuvants described above, immunogenicity of peptides of the current invention can be enhanced by attachment of the peptides to proteosomes or by addition of a cystein residue. For attachment to proteosomes, a spacer, such as a CYGG (SEQ ID NO: 11) spacer, and a lauroyl group can be attached to the peptide's amino terminal end (Bio-Synthesis, Lewisville, TX). The lauroyl group enhances the hydrophobic complexing of peptide groups to proteosomes (Lowell, G. H., et al., "Proteosomes, hydrophobic anchors, iscoms, and liposomes for improved presentation of peptide and protein vaccines," in: *New Generation Vaccines*, Woodrow, G.M., Levine, M.M. (Ed.), Marcel Dekker, Inc., New York, pp. 141-160 (1990); Lowell, G. H., et al., "Peptides bound to proteosomes via hydrophobic feet become highly immunogenic without adjuvants," *J. Exp. Med.* 167:658 (1988); and Zollinger, W. D., et al., "Complex of meningococcal group B polysaccharide and type 2 outer membrane protein immunogens in man," *J. Clin. Invest.* 63:836 (1979)). A cysteine group on the other hand, such as the cysteine in the CYGG spacer described above, enhances the immunogenicity of the peptide (Lowell, G. H., et al., (1990)). Proteosomes can be prepared from the outer membrane complex vesicles from Group B meningococci, strain 99M as described by Zollinger (Zollinger, et al., (1990)). Synthetic lipopeptides can be complexed to proteosomes on a 1:1 (w/w) ratio by combining the components in the presence of detergent. The detergent can be removed by extensive dialysis (Lowell, G. H., et al., (1988)).

Please amend the paragraphs between page 48, line 22 and page 51, line 22 as follows:

Analysis of type 6B PsaA. Genomic DNA was partially digested by *Sau3AI* was ligated to *Bam*HI-digested pUC18 and used to transform *E. coli* DH5a. Recombinant colonies were selected for resistance to ampicillin and the formation of white colonies in the presence of

isopropyl- β -galactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside. Colony immunoblot screening (using anti-PsaA MAb) of approximately 2,500 colonies yielded two positive clones, which were selected, purified, and rescreened by Western blot analysis using the same MAb. They both expressed a protein reactive with MAb to PsaA and which migrated in SDS-PAGE with the expected molecular mass of approximately 37-kDa. One was selected for continued study and was designated pSTR6. Limited restriction enzyme analysis of DNA from the recombinant plasmid showed that the positive clone contained an insert that was 3.5 kb with sites for enzymes *Cla*I, *Eco*RI, and *Hind*III. To localize the PsaA coding region, the insert was double digested with *Sst*I (multiple cloning site in vector) and *Hind*III. The resultant fragments were ligated into pUC18 and transformed into *E. coli* DH5 α . This generated a recombinant containing an insert of about 1.3 kb in size. The resultant subclone pSTR6y, when analyzed by SDS-PAGE and Western blot using anti-PsaA MAb, was shown to express full length PsaA immuno-reactive protein. The complete nucleotide sequence on both strands of the 1.3-kb insert was determined by cycle sequencing of the plasmid subclone using oligonucleotide primers complementary to the sequence. These were made as sequence information became available. The nucleotide sequence of the entire streptococcal insert is set forth in the Sequence Listing as SEQ ID NO:1. The single open reading frame (ORF) present, beginning at nucleotide 189 and ending at nucleotide 1,117, encodes the PsaA gene sequence. This ORF is 930 nucleotides long and when amplified and subcloned into vector systems such as pGEM (Promega, Madison, Wis.) and BAC-to-BACTM expression system (Bethesda Research Laboratories, Gaithersburg, Md.) expresses full-length PsaA, reactive with anti-PsaA MAb antibodies. This ORF encodes a peptide of 309 amino acids with a deduced molecular weight of 34,598 and an isoelectric point of 5.23. Analysis of the peptide using the algorithm of Kyte et al., 1982, ("A simple method for displaying the hydropathic character of a protein," *J. Mol. Biol.* 157:105-132.) shows that the peptide contains a major hydrophobic region of 20 amino acids which encodes a putative leader sequence. This leader contains the consensus sequence for signal peptidase cleavage (LXXC) (SEQ ID NO: 12). Removal of this leader would result in a

peptide of molecular mass 32,465 with a predicted isoelectric point of 4.97. A consensus sequence for a ribosomal binding site (Shine et al., 1974, "The 3'-terminal sequence of *E. coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosomal binding sites," *Proc. Natl. Acad. Sc.. USA* 71:1324-1346) is located 5 nucleotides upstream of the ATG start codon.

Comparison of the serotype 6B sequence with streptococcal homologs. Comparison of the serotype 6B PsaA nucleotide sequence (Bilofsky et al., 1988, A GenBank genetic sequence database, *Nucleic Acids Res.* 16:1861-1864) (GenBank accession number U53509) and its flanking regions with the previously published strain R36A PsaA sequence (Sampson et al., 1994, "Cloning and nucleotide sequence analysis of PsaA, the *Streptococcus pneumoniae* gene encoding a 37kilodalton protein homologous to previously reported *Streptococcus* sp. adhesins " *Infect. Immun* 62:319-324) shows the differences between the nucleotide sequences. The computed homology between the two sequences is 74%. Major areas of discord are in regions upstream and downstream of the ORF and in the initial 60 nucleotide which encode the putative signal peptide. When the two PsaA coding sequences are compared, the sequence homology increases to 78%. Serotype 6B sequence was also compared to the PsaA DNA sequence for another vaccine serotype, serotype 2, which was recently submitted to GenBank (Accession number U40786). Computer analysis of these two sequences shows that they are very similar, with computed DNA homology percentages of 99% between the two PsaA DNA sequences. There are eight single base differences between the two sequences. A comparison of serotype 2 and 6B PsaAs shows almost complete identity: the computed similarity value is 99.3. The eight base difference at the nucleotide level translated into a difference at the peptide level of six amino acids with two of the changes resulting in conservative substitutions. Further analyses and comparisons of the serotype 6B sequence to the other five GenBank PsaA homologues from viridans *Streptococci* and *E. faecalis* (Fenno et al., 1989, "Nucleotide sequence analysis of a type I fimbrial gene of *Streptococcus sanguis* FW213." *Infect. Immun.* 57:3527-3533; Sampson et al., 1994, "Cloning and nucleotide sequence analysis of PsaA, the *Streptococcus pneumoniae* gene encoding a 37-kilodalton protein homologous to previously reported *Streptococcus* sp.

adhesins," *Infect. Immun.* 62:319-324; Ganeshkumar et al., 1991. "Nucleotide sequence of a gene coding for a saliva-binding protein (SsaB) from *Streptococcus sanguis* 12 and possible role of the protein in coaggregation with actinomyces." *Infect. Immun.* 59:1093-1099, Kolenbrander et al., 1994. "Nucleotide sequence of the *Streptococcus gordonii* PK488 coaggregation adhesin gene *scaA* and ATP binding cassette." *Infect. Immun.* 62:4469-4480; and Lowe et al., 1995. "Cloning of an *Enterococcus faecalis* endocarditis antigen: homology with some adhesins from oral streptococci." *Infect. Immun.* 63:703-706) revealed significant sequence similarity between them. Sequence identities were 81%, 81%, 77%, 82%, and 57%, respectively, for PsaA (*S. pneumoniae* strain R36A), SsaB (*S. sanguis*), FimA (*S. parasanguis*), ScaA (*S. gordonii*) and EfaA (*E. faecalis*). Additionally, all six sequences showed great similarity in organization. They have a hydrophobic leader peptide containing the prolipoprotein consensus sequence LXXC (for signal peptidase II cleavage) (SEQ ID NO: 12) within the first 17-20 amino acids. This N-terminal leader sequence appears to represent the area of greatest variability. It is followed by a region of high similarity from amino acids 36 to 150. The region from 150 to 198 is a variable region and is followed by another conserved region (198 to 309).